The family Noctuidae includes some of the most damaging agricultural pests worldwide. In India, two species of Helicoverpa and one species of Heliothis have been recorded, viz. Helicoverpa armigera (Hubner), Helicoverpa assulta Guenee and Heliothis peltigera (Denis and Schiffermuller)\(^1\). The three species were originally included under the same genus Heliothis before armigera and assulta were transferred to the new genus Helicoverpa\(^2\). Here we report a molecular tool for distinguishing the two species of Helicoverpa.

H. armigera and H. assulta are found throughout Africa, Asia, parts of Australasia and the South Pacific. H. armigera is polyphagous\(^3\) and insecticide-resistant\(^4\). H. assulta is an oligophagous pest. Its principal hosts are tobacco, chilli bell pepper and wild hosts in the genus Datura\(^5\). There are no reports of control failures or insecticide resistance in this species in the Indian subcontinent. However, control failures of H. assulta on capsicum have been attributed to insecticide resistance in South Korea\(^6\).

During field collections of H. armigera eggs and larvae in India, it was common to come across mixed populations of H. armigera and H. assulta on tobacco and on wild hosts in the family Solanaceae. Between April and June, mixed populations of H. armigera and H. assulta occurred on Datura and other solanaceous host plants like capsicum and chilli. Light traps operating between August 1974 and May 1987 at ICRISAT, Patan- cheru, indicated that H. armigera was at least 100 times more abundant than H. assulta over most of the cropping season\(^7,8\). Peak catches of H. assulta were confined from August to October, which also coincided with those of H. armigera on cotton. In view of these overlapping populations, it may possibly be confusing to distinguish between H. armigera and H. assulta. H. assulta is considered a minor pest, but its importance may be underestimated because of the similarity of both larvae and moths to those of H. armigera\(^9\). It requires the services of a trained entomologist to differentiate the two species in the adult stage, applying taxonomic keys as described by Hardwick\(^6\) and Mathews\(^8,9\). Eggs and neonates are virtually indistinguishable in mixed populations. A molecular, stage-independent tool based on the mitochondrial genome is being proposed for distinguishing the two species of Helicoverpa.

H. assulta larvae were collected from Datura in and around cotton fields of Naggur. H. armigera were collected as eggs from cotton. Larvae were reared till pupation on semi-synthetic diet\(^10\). The emerging moths were identified using the taxonomic key described earlier\(^3,8,9\). Genomic DNA was isolated from the thorax of female moths using the protocol described by Zhang et al.\(^11\). The mid CO-I (cytochrome oxidase) region has a high functional significance and was therefore chosen for the study. Primers were designed to amplify the specific CO-I region\(^12\): C1-J-2090 and C1-N-2659, whose significance is not being speculated herein. The region sequenced in this study was capable of being selectively restricted in H. armigera with eight restriction enzymes, with Rsal being one of them. Rsal was chosen for the study as it demonstrated the ability of cutting the sequenced region approximately in the centre, resulting in two fragments of approximately 333 and 265 bp each, as shown in Figure 1. Its recognition site is masked in H. assulta, which is responsible for the absence of restriction digestion.

PCR–RFLP as a tool demonstrates reliable differentiation of the two species. It offers support to conventional taxonomic differentiation based on morphological features. Both techniques, however, require skill and expertise. Mutilated museum specimens that are difficult to study using the taxonomic key can be readily identified with this tool. This method can be used as a molecular tool for distinguishing H. armigera and H. assulta from each other especially in ecosystems that harbour a mixture of both species. 

**Figure 1.** Restriction digestion of PCR-amplified partial CO-I region of H. armigera and H. assulta with Rsal. Lanes 1, 9, 100 bp MW ladder; lanes 2, 3, H. armigera PCR product digested with Rsal; lane 4, H. armigera PCR product undigested control; lanes 5, 6, H. assulta PCR product digested with Rsal; lane 7, H. assulta PCR product undigested control; lane 8, Negative control.
Regeneration of plants from root explant of two Indian cultivars of 
\textit{Brassica campestris} L. through somatic embryogenesis

\textit{Brassica campestris} L. is cultivated worldwide as an important edible and industrial oilseed crop. Though organogenic shoot regeneration from callus\textsuperscript{1,2}, leaf disc\textsuperscript{3}, cotyledon\textsuperscript{4,5}, cotyledonary petiole\textsuperscript{6,7}, hypocotyl\textsuperscript{4,15}, isolated protoplasts\textsuperscript{16,17}, anther\textsuperscript{18} and isolated microspore\textsuperscript{19-21} of \textit{Brassica campestris} L. has been reported, regeneration through embryogenesis has not yet been reported in this species. Plant regeneration from root explant in Cruciferae has been reported in \textit{B. oleracea}\textsuperscript{22} and \textit{Arabidopsis thaliana}\textsuperscript{23-25}, but not from \textit{B. campestris}\textsuperscript{26}. Embryogenic plant is considered to be most desirable for \textit{Agrobacterium}-mediated transgenic plant production. The present correspondence describes efficient plant regeneration protocol through embryogenesis from root explants of two Indian cultivars of \textit{B. campestris}, i.e. cv. B-54 (Agrani) and cv. B-9 (Benoy). Henceforth the two cultivars will be designated as B-54 and B-9 respectively, in the text.

Medium – BS1: Half concentration of MS salts and vitamins\textsuperscript{27}, 10 g l\textsuperscript{-1} sucrose, 0.8% agar, pH 5.8; BS2 to BS4: As Mant and Browse\textsuperscript{28}, but hormone requirement of respective medium is depicted in Table 1; MS-H: MS salts and vitamins, 20 g l\textsuperscript{-1} sucrose, pH 5.8, 1% agar. All the media were supplemented with 3 ml l\textsuperscript{-1} Miller’s solution\textsuperscript{29}. In two other experiments, either 2.5 mg l\textsuperscript{-1} silver thiosulphate or 3.3 mg l\textsuperscript{-1} AgNO\textsubscript{3} was used. All hormones, silver thiosulphate and AgNO\textsubscript{3} were filter-sterilized and added to autoclaved media before plating. Silver thiosulphate or AgNO\textsubscript{3} was not added to MS-H medium.

Surface-sterilized seeds (30 min in liquid detergent Teepol, washed in tap water, 5 min in 0.2% HgCl\textsubscript{2} solution, washed in sterile water six times) were germinated on BS1 medium in petri dishes (90 mm Ø) over the embryo induction medium (BS2); and cultured at 26 ± 1°C in a 16/8 h light/dark photoperiod (light intensity 3000 lx) for 7–14 days. Subsequently, the explants were transferred to the embryo elongation medium (BS3) and cultured for another 10–14 days. The embryogenic root segments were transferred to embryo maturation medium (BS4). After the visible appearance of shoots in these embryos, they were transferred to MS-H medium for rooting and further development. The rooted plants were hardened and transferred to field, according to Mandal and Sikdar\textsuperscript{30}.

Table 1 depicts the high responsive media used in different stages of embryo development. In the embryo induction phase, BS2 III and BS2 XII gave the best response for BS-54 (88.2 %) and B-9 (90.8%) cultivars respectively (Table 2). In the elongation phase BS3-type medium, i.e. BS3 II, III, VI and IX for B-54 and BS3 XVII and XVIII for B-9 could show high response in developing green embryos (0–12/root segment, average 3.52 for B-54 to 21-day-old seedlings and wounded by squeezing firmly with fine forceps at 2–5 mm intervals and spread uniformly (25–30 segments/90 mm Ø plate) over the embryo induction medium (BS2); and cultured at 26 ± 1°C in a 16/8 h light/dark photoperiod (light intensity 3000 lx) for 7–14 days. Subsequently, the explants were transferred to the embryo elongation medium (BS3) and cultured for another 10–14 days. The embryogenic root segments were transferred to embryo maturation medium (BS4). After the visible appearance of shoots in these embryos, they were transferred to MS-H medium for rooting and further development. The rooted plants were hardened and transferred to field, according to Mandal and Sikdar\textsuperscript{30}.

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